THE PRIMARY STRUCTURE OF PROTEIN L1 FROM THE LARGE RIBOSOMAL SUBUNIT OF *ESCHERICHIA COLI*

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1. Introduction

Protein L1 is a basic protein from the large *Escherichia coli* ribosomal subunit [1]. It binds to the 23 S RNA and protects 148 nucleotides from nuclease digestion located between position 2000 and 2500 [2,3]. Immune electron microscopy has shown that there are three binding sites on the surface of the 50 S subunit for specific antibodies raised against L1 (reviewed [4,5]). Protein L1 (amongst other proteins) can be crosslinked to EF-Tu. The formation of this crosslink is dependent on the presence of aminoacyl-tRNA and non-hydrolyzable GTP [6]. In this paper we describe the complete primary structure of protein L1.

2. Materials and methods

Protein L1 was isolated from *E. coli* strain K as in [7] and was provided by Dr H. G. Wittmann.

2.1. *Enzymic digestions*

Tryptic digestions were performed with TPCK (1-chloro-4-phenyl-3-tosyl-amidobutan-2-one) pretreated enzyme at pH 8 for 4 h at 37°C. Digestion with thermolysin (Serva, Heidelberg) was made at pH 8 for 90 min at 50°C, and with *Staphylococcus aureus* protease from Miles Biochemicals (Frankfurt/Main) in 0.05% ammonium acetate buffer (pH 4.0) for 48 h at 37°C. For details see [8].

2.2. *Modified enzymic digestions*

Reversible modification of the protein was achieved by protecting the ε-amino groups of the lysine residues with ETPA (exo-*cis*-3,6-endoxo-Δ^4-tetrahydropthalic anhydride) followed by tryptic digestion [9]. Modification of the arginine residues with 1,2-cyclohexandione and tryptic digestion was made by the method in [10].

2.3. *Isolation of peptides*

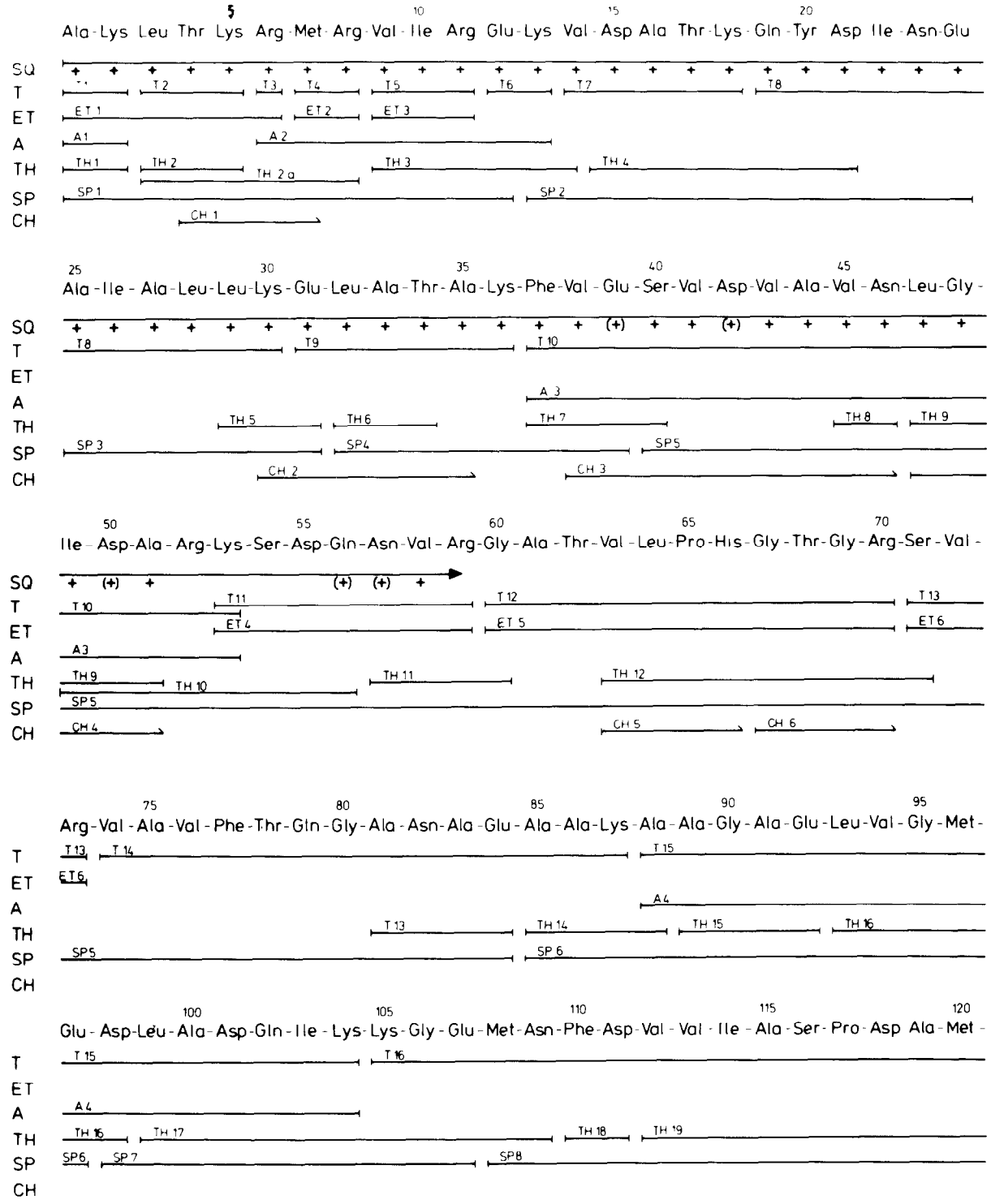
The separation of peptides on a preparative scale was achieved either by gel filtration on Sephadex G-50 (superfine, 140 × 1 cm) in 10% acetic acid [8] or on a microcolumn of Dowex M71 (0.3 × 10 cm) at 55°C using pyridine formate gradients [8,11]. The fractions were analysed either photometrically at 280 nm or by spotting aliquots of every second fraction onto cellulose thin-layer plates. Peptides which were unresolved at this stage were subjected to further separation procedures, using either preparative chromatography, electrophoreses or the fingerprint technique on thin-layer plates [8].

2.4. *Amino acid analyses*

All analyses of protein samples and peptides were performed on a Durrum D-500 analyser. For estimation of the cysteine content the protein was oxidized with performic acid before acid hydrolysis. The presence of tryptophan was tested for by spraying the fingerprint plates with *p*-aminobenzaldehyde [12] and by amino acid analysis after hydrolysis with methane sulfonic acid [13].

2.5. *Methods for sequencing*

Automated and manual techniques were used for the determination of amino acid sequences:


Ala-Lys-Leu-Thr-Lys-Arg-Met-Arg-Val-Ile-Arg-Glu-Lys-Val-Asp-Ala-Thr-Lys-Gln-Tyr-Asp-Ile-Asn-Glu
SQ
T
ET
A
TH
SP
CH
T1
T2
T3
T4
T5
T6
T7
T8
ET1
ET2
ET3
A1
A2
TH1
TH2
TH2a
TH3
TH4
SP1
SP2
CH1
Ala-Ile-Ala-Leu-Leu-Lys-Glu-Leu-Ala-Thr-Ala-Lys-Phe-Val-Glu-Ser-Val-Asp-Val-Ala-Val-Asn-Leu-Gly-
T8
T9
T10
A3
TH5
TH6
TH7
TH8
TH9
SP3
SP4
SP5
CH2
CH3
Ile-Asp-Ala-Arg-Lys-Ser-Asp-Gln-Asn-Val-Arg-Gly-Ala-Thr-Val-Leu-Pro-His-Gly-Thr-Gly-Arg-Ser-Val-
T10
T11
T12
T13
ET4
ET5
ET6
A3
TH9
TH10
TH11
TH12
SP5
CH4
CH5
CH6
Arg-Val-Ala-Val-Phe-Thr-Gln-Gly-Ala-Asn-Ala-Glu-Ala-Ala-Lys-Ala-Ala-Gly-Ala-Glu-Leu-Val-Gly-Met-
T13
T14
T15
ET6
A4
T13
TH14
TH15
TH16
SP5
SP6
CH
Glu-Asp-Leu-Ala-Asp-Gln-Ile-Lys-Lys-Gly-Glu-Met-Asn-Phe-Asp-Val-Val-Ile-Ala-Ser-Pro-Asp-Ala-Met-
T15
T16
A4
TH16
TH17
TH18
TH19
SP6
SP7
SP8
CH

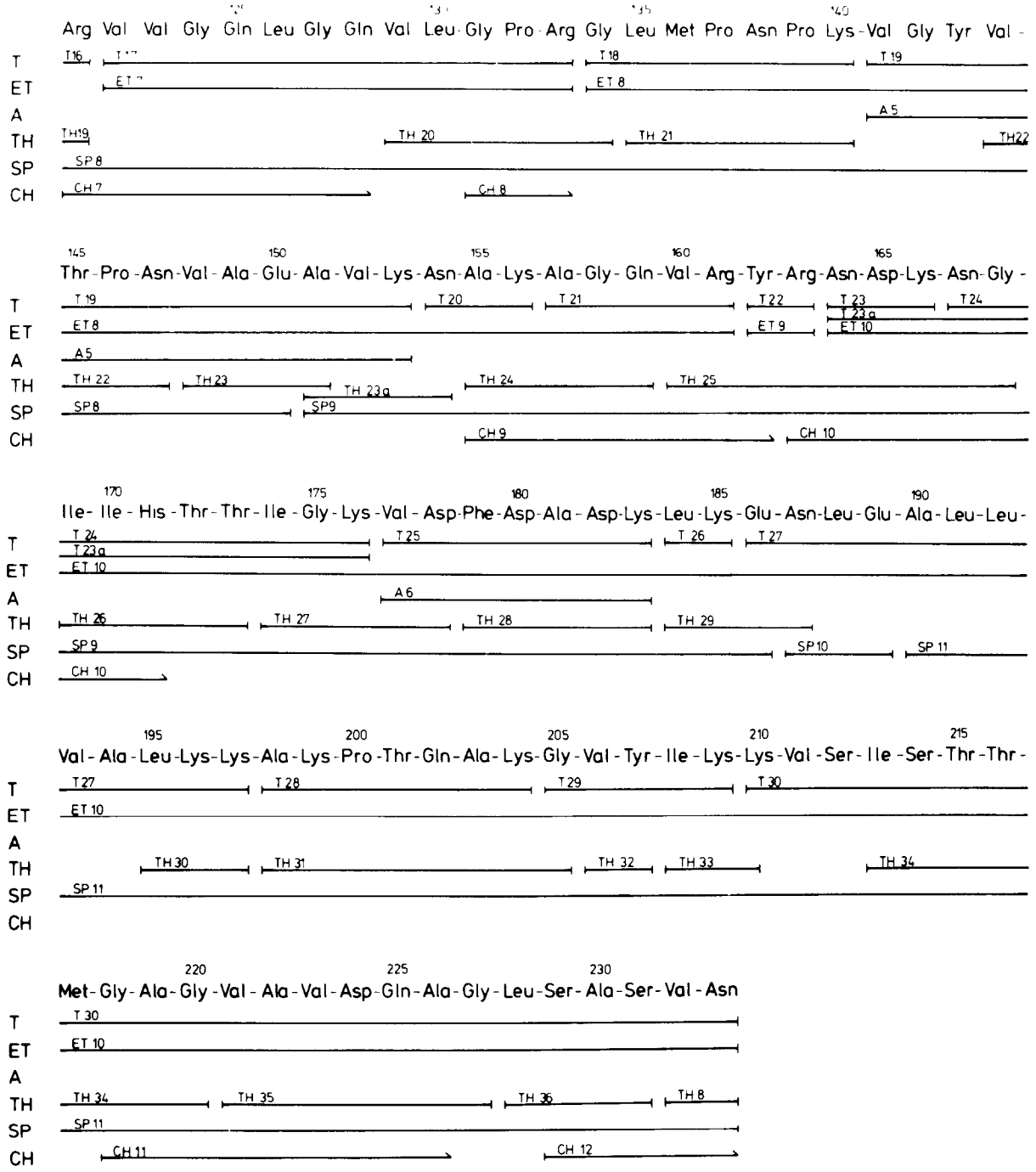


Fig.1. The primary structure of L1 from *Escherichia coli*. (SQ) Liquid-phase Edman degradation in a modified sequenator. (∓) Residues unambiguously identified by thin-layer chromatography. (T) Tryptic peptides. (ET) Tryptic peptides after blocking the lysines by ETPA. (A) Tryptic peptides after blocking the arginines by 1,2-cyclohexandione. (SP) Peptides from digestion with *Staphylococcus* protease. (TH) Peptides from thermolysin digestion. (CH) Peptides from chymotrypsin digestion.

1. Automatic Edman degradation [14] of intact protein in an improved Beckman sequenator [15].
2. Manual Edman degradation combined with dansylation of the free N-terminal amino acid residue after each degradation step [16].
3. Manual Edman-type degradation using a double coupling method with 4-*NN*-dimethylamino-azobenzene-4′-isothiocyanate [17].

The determination of aspartic acid, glutamic acid and their corresponding amides was achieved by converting the released 2-anilino-5-thiazolinone derivatives (method 2) to the corresponding phenylthiohydantoin derivatives followed by chromatographic identification [15]. Ambiguities in the identification of dansylated amino acids (2) were resolved by liberation of the free amino acids from their 2-anilino-5-thiazolinones or phenylthiohydantoin derivatives by hydrolysis followed by amino acid analysis.

3. Results and discussion

Protein L1 was digested with various enzymes and the resulting peptides were isolated and sequenced. The results are summarized in fig.1. Automatic Edman degradation was performed with the intact protein in an improved Beckman sequenator. Comparison of the published N-terminal sequence [18] with the N-terminal peptides shown in fig.1 gave a good agreement. Combination of all the results (which will be detailed elsewhere) led to the complete primary structure determination of L1 as shown in fig.1. Our structure of protein L1 fully agrees with the data obtained by determining the DNA sequence of the L1 gene in Dr M. Nomura's laboratory (personal communication).

Protein L1 consists of 233 amino acid residues with the following amino acid composition: Asp_{14}, Asn_{12}, Thr_{13}, Ser_{8}, Glu_{11}, Gln_{9}, Pro_{7}, Gly_{20}, Ala_{33}, Val_{29}, Met_{6}, Ile_{11}, Leu_{17}, Tyr_{3}, Phe_{4}, His_{2}, Lys_{23} and Arg_{11}. The amino acid composition leads to mol. wt 24 602, which is in good agreement with that determined by hydrodynamic methods, namely 25 000 (L. Giri, personal communication) and it is 8% lower than that determined by SDS–gel electrophoresis [19].

During our sequence work there was no hint of the presence of methylated amino acids as reported [20]. For instance no unusual peaks were obtained during the amino acid analyses of the protein or the peptides, and no unusual chemical behaviour was observed for the amino acid derivatives obtained by different manual degradation methods. This can be explained by assuming that methylation of particular amino acid residues occur at such a low amount that it cannot be detected by the methods described here.

Acknowledgement

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